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Stolc et al.

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(54) **RAPID POLYMER SEQUENCER**

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G01N 33/48 (2006.01)
G06F 15/00 (2006.01)

(52) **U.S. Cl.**
USPC **702/19**; 712/220

(58) **Field of Classification Search**
None
See application file for complete search history.

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Final Rejection, mailed Jul. 28, 2008, in parent case, U.S. Appl. No. 10/885,537, filed Jun. 24, 2004, issued May 24, 2011 as patent No. 7,949,472.
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(57) **ABSTRACT**

Method and system for rapid and accurate determination of each of a sequence of unknown polymer components, such as nucleic acid components. A self-assembling monolayer of a selected substance is optionally provided on an interior surface of a pipette tip, and the interior surface is immersed in a selected liquid. A selected electrical field is impressed in a longitudinal direction, or in a transverse direction, in the tip region, a polymer sequence is passed through the tip region, and a change in an electrical current signal is measured as each polymer component passes through the tip region. Each of the measured changes in electrical current signals is compared with a database of reference electrical change signals, with each reference signal corresponding to an identified polymer component, to identify the unknown polymer component with a reference polymer component. The nanopore preferably has a pore inner diameter of no more than about 40 nm and is prepared by heating and pulling a very small section of a glass tubing.

18 Claims, 9 Drawing Sheets

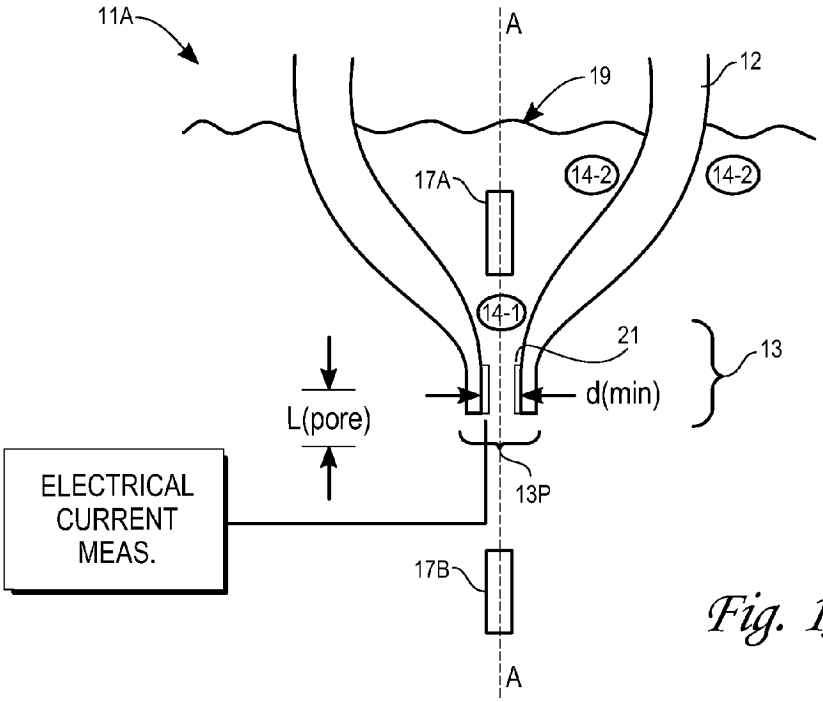
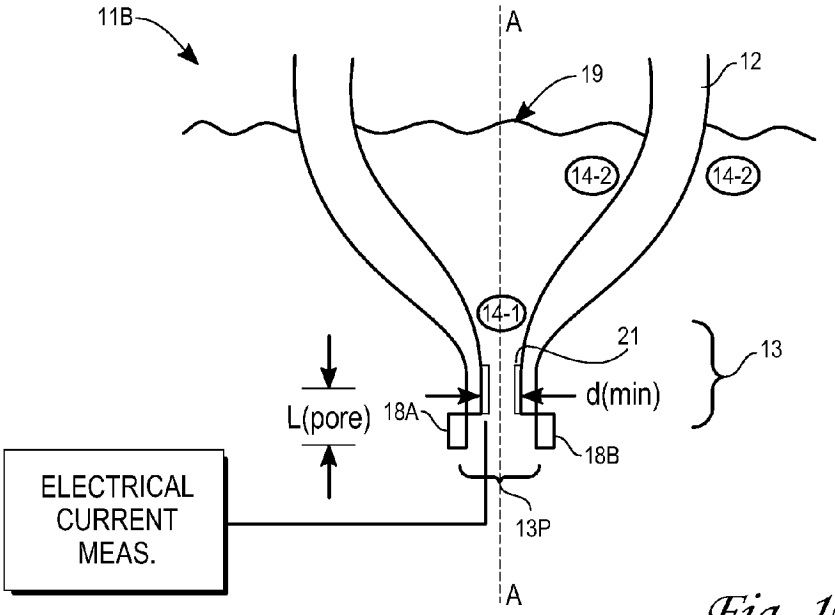

11A
A
19
12
17A
14-2
14-2
14-1
21
13
d(min)
L(pore)
13P
ELECTRICAL
CURRENT
MEAS.
17B
A

Fig. 1A


11B
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d(min)
L(pore)
18A
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13P
ELECTRICAL
CURRENT
MEAS.
A

Fig. 1B

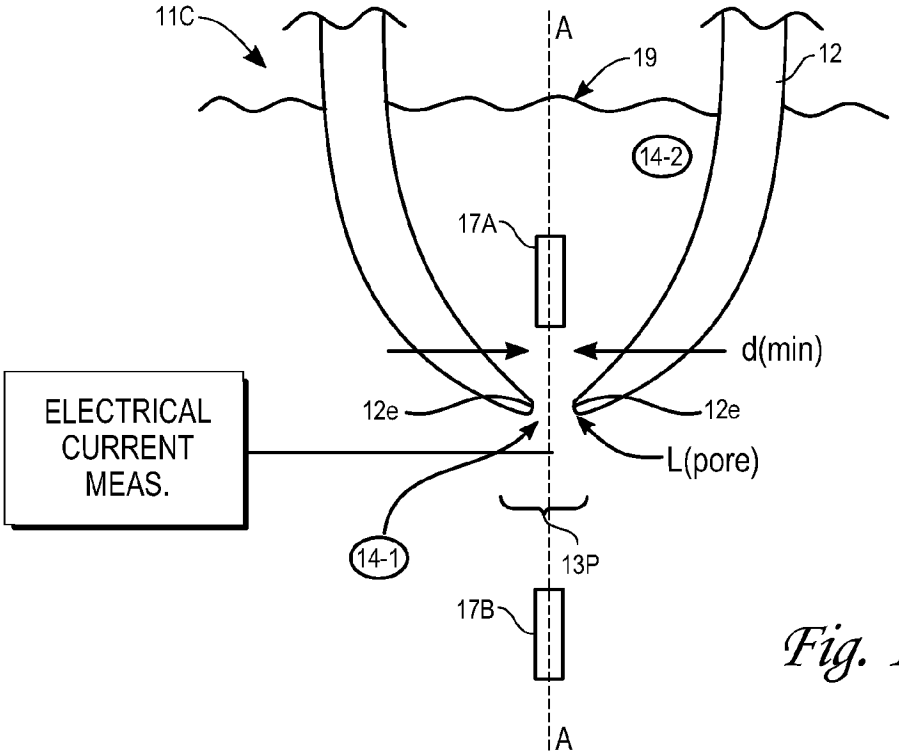

Fig. 1C

Fig. 2A (Polymer Absent)

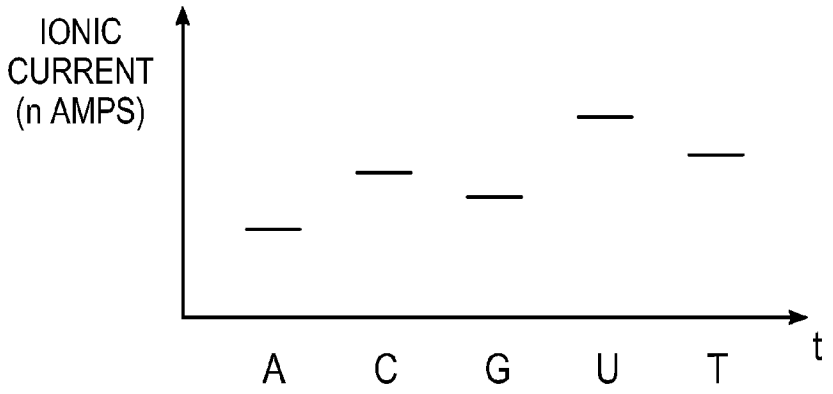

Fig. 2B

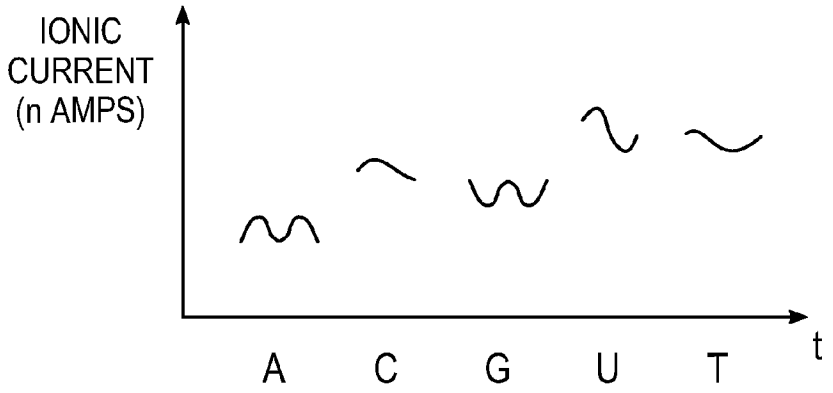

Fig. 2C

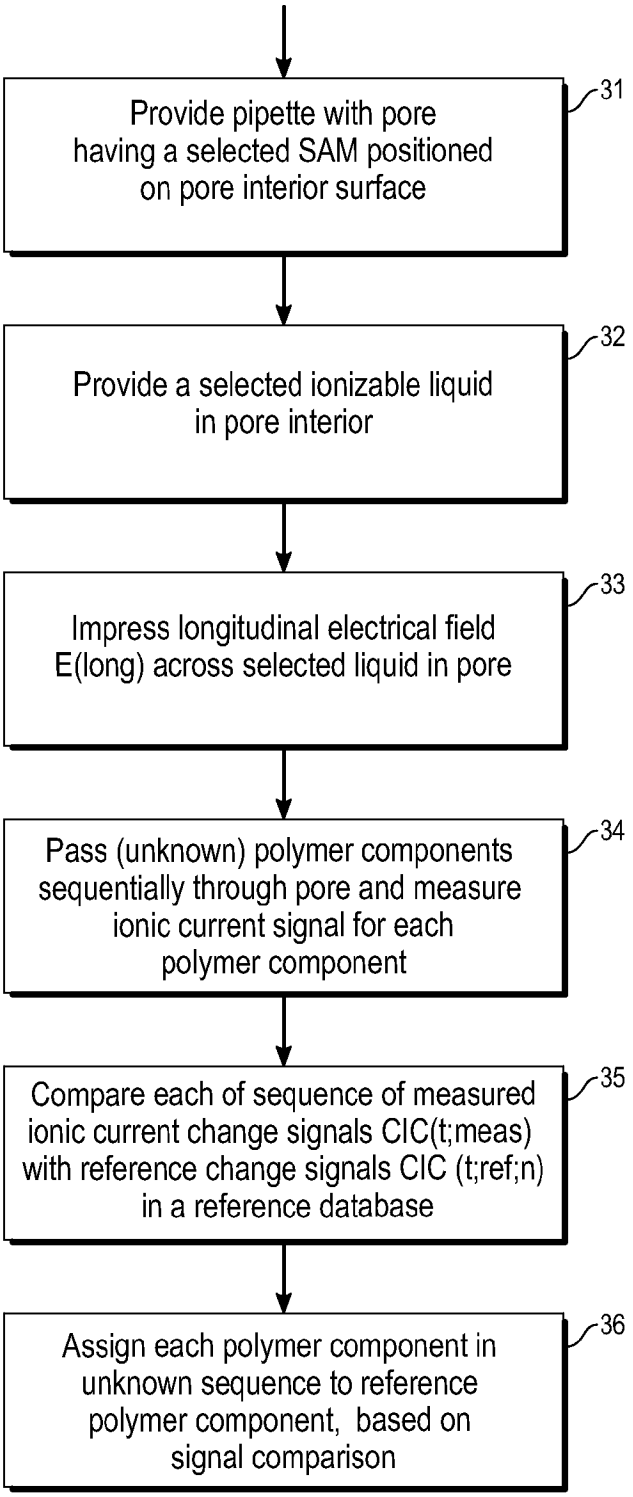
Provide pipette with pore having a selected SAM positioned on pore interior surface
31
Provide a selected ionizable liquid in pore interior
32
Impress longitudinal electrical field E(long) across selected liquid in pore
33
Pass (unknown) polymer components sequentially through pore and measure ionic current signal for each polymer component
34
Compare each of sequence of measured ionic current change signals CIC(t;meas) with reference change signals CIC (t;ref;n) in a reference database
35
Assign each polymer component in unknown sequence to reference polymer component, based on signal comparison
36

Fig. 3

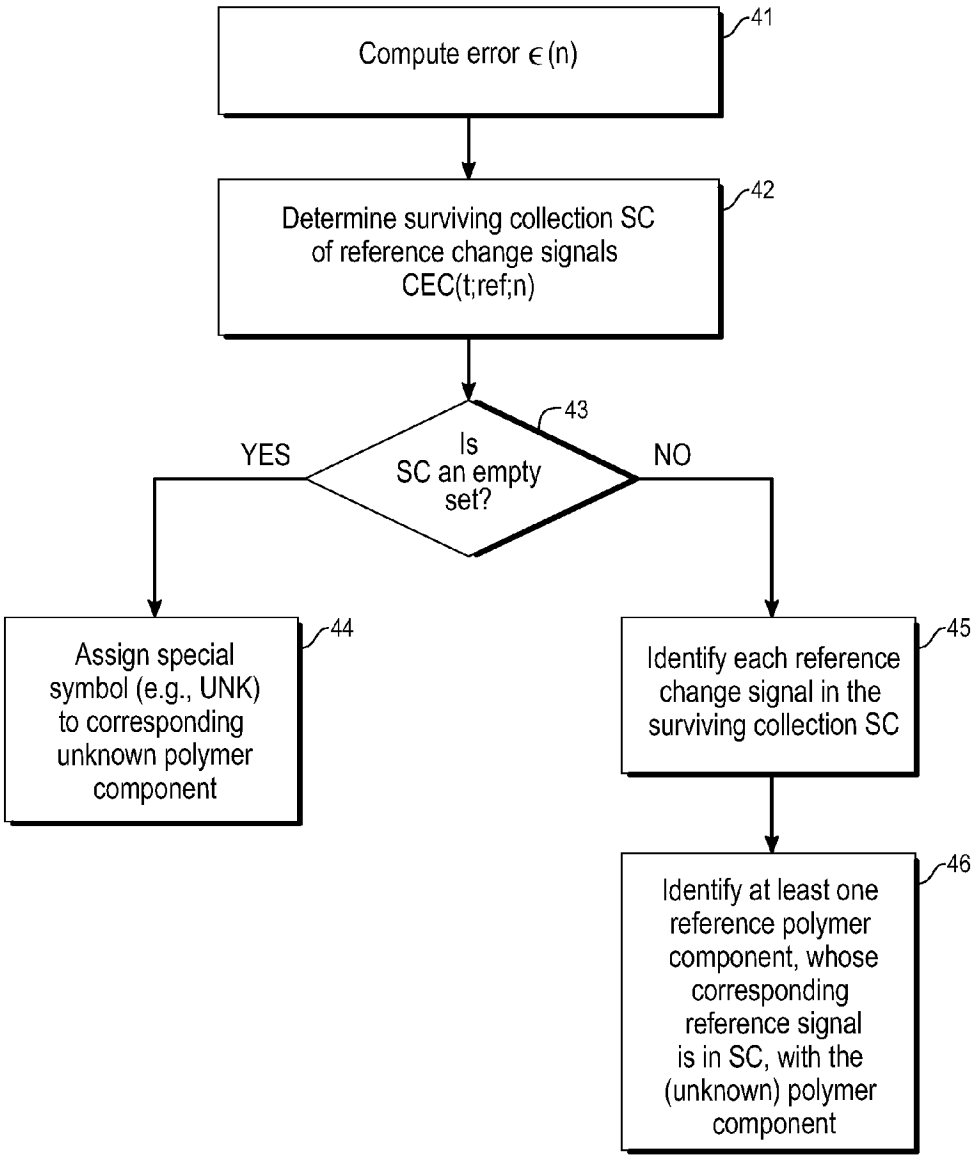
Compute error ϵ (n)
41
Determine surviving collection SC of reference change signals CEC(t;ref;n)
42
Is SC an empty set?
43
YES
NO
Assign special symbol (e.g., UNK) to corresponding unknown polymer component
44
Identify each reference change signal in the surviving collection SC
45
Identify at least one reference polymer component, whose corresponding reference signal is in SC, with the (unknown) polymer component
46

Fig. 4

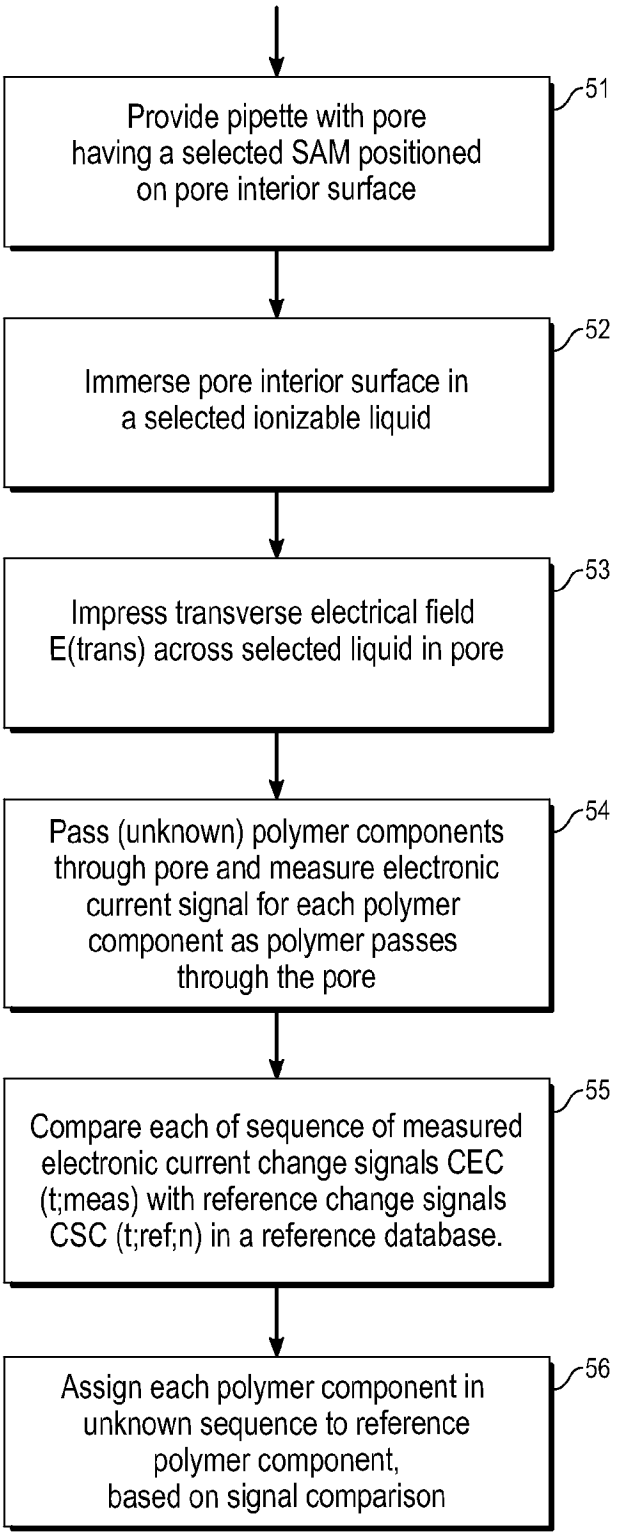
Provide pipette with pore having a selected SAM positioned on pore interior surface
51
Immerse pore interior surface in a selected ionizable liquid
52
Impress transverse electrical field E(trans) across selected liquid in pore
53
Pass (unknown) polymer components through pore and measure electronic current signal for each polymer component as polymer passes through the pore
54
Compare each of sequence of measured electronic current change signals CEC (t;meas) with reference change signals CSC (t;ref;n) in a reference database.
55
Assign each polymer component in unknown sequence to reference polymer component, based on signal comparison
56

Fig. 5

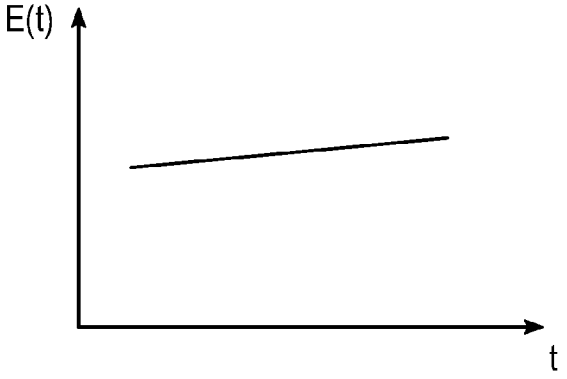

Fig. 6A

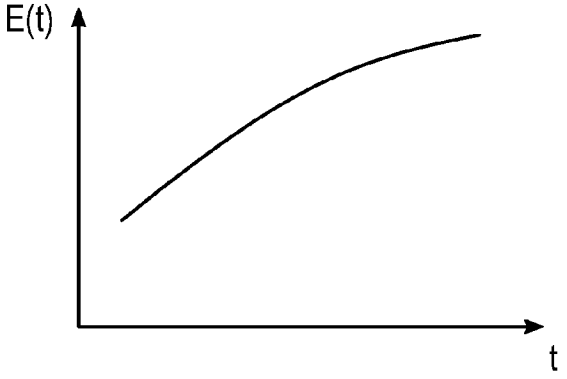

Fig. 6B

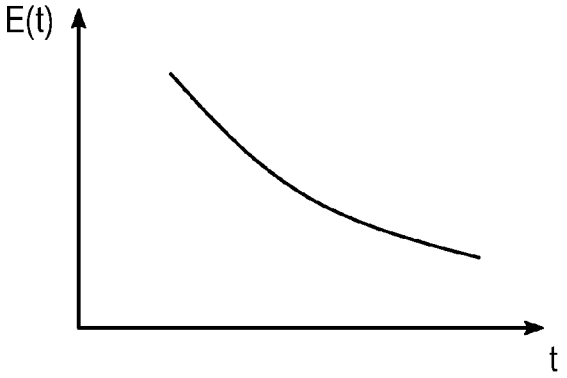

Fig. 6C

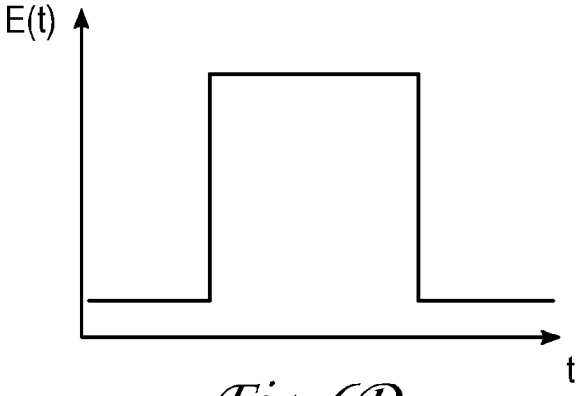

Fig. 6D

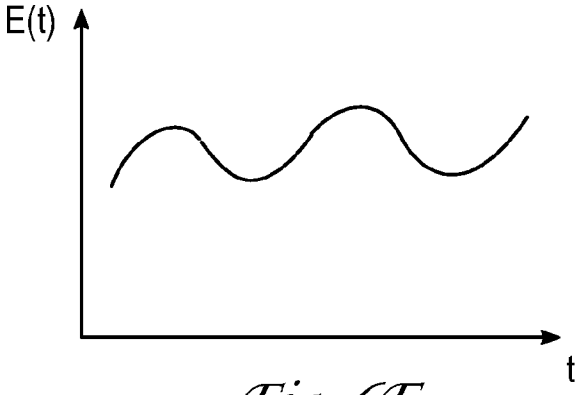

Fig. 6E

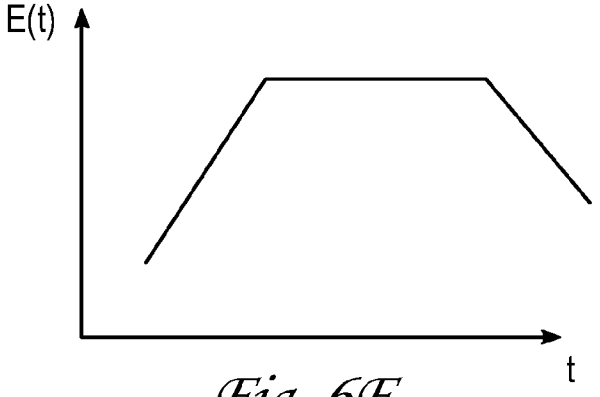

Fig. 6F

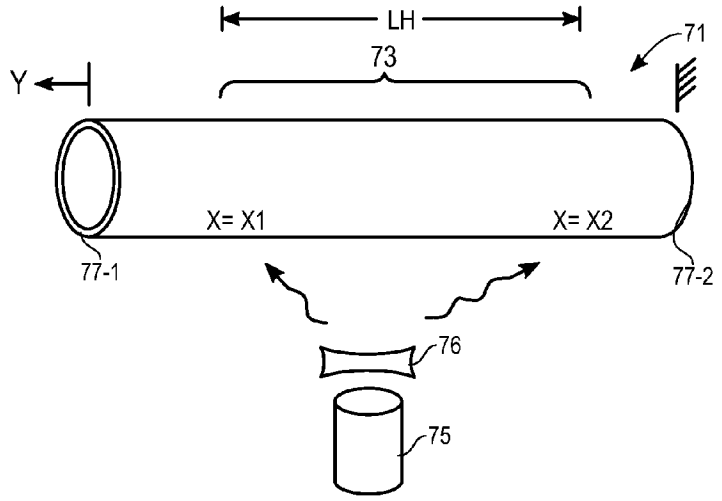

Fig. 7A

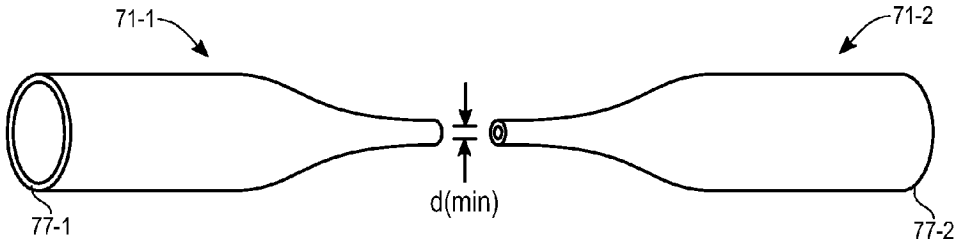

Fig. 7B

RAPID POLYMER SEQUENCER

ORIGIN OF THE INVENTION

The invention described herein was made, in part, by an employee of the United States Government and may be manufactured and used by or for the Government for governmental purposes without the payment of any royalties thereon or therefor.

TECHNICAL FIELD

The present invention is a method and system for rapidly and accurately determining an ordered sequence of molecular units, such as bases in a nucleic acid, such as DNA or RNA, and for fabricating a nanopore system to facilitate the sequencing.

BACKGROUND OF THE INVENTION

Nanofabrication techniques offer the possibility to create solid state pores or apertures with diameters and lengths similar to diameters and lengths of single nucleotides or proteins. Solid state nanopores permit use of non-physiological conditions for structural manipulation of biopolymers, such as non-neutral pH levels, high temperatures and/or high voltage differences. Use of a solid state substrate will allow a more straightforward manipulation of surface chemistry in the pore, which may be critical to fine-tune the rate of nucleic acid translocation or the degree of ionic current reduction associated with passage of a polymer, such as a poly-nucleotide through a nanopore.

Kasianowicz et al, in "Characterization of individual polynucleotide molecules through a membrane channel," Proc. Nat. Acad. Sci. vol. 93 (1996) 195-223, have used a pore of diameter about 1.5 nanometers (nm) in the bacterial α-hemolysin ion channel protein, and have applied an electrical field to drive a negatively charged polynucleotide through the pore from one side to the other, which transiently reduces ionic conductance through the pore. Akeson et al, in "Microsecond Time Scale Discrimination Among Polycytidylic Acis in Homopolymers or as Segments Within Single RNA Molecules," Biophys. Jour. Vol. 77 (1999) 3227-3233, have shown that polynucleotides of different lengths can be discriminated by time duration of translocation as the nucleotide sequence passes through a pore. Translocation of different nucleotide homopolymers reduces ionic conductance of α-hemolysin by characteristic amounts, which suggests that the individual nucleotides in a heteropolymer could be identified, if passed through a nanopore of appropriate dimensions and composition. However, α-hemolysin has a pore length as long as a sequence of about 20 nucleotides so that discrimination between individual nucleotides using α-hemolysin is not possible.

What is needed is a system that provides rapid and accurate identification of ordered components of a nucleic acid, protein or similar polymer, at rates up to and above one component per μsec. Preferably, the approach should adequately discriminate between the different ordered components present in the polymer and provide accurate ordering, with an acceptable error rate that is controllable by varying the rate at which the polymer components pass through and is read by the system.

SUMMARY OF THE INVENTION

These needs are met by the invention, which provides a system and associated method that relies upon a pore at a

pipette tip, having a pore diameter as small as 1-40 nm, preferably containing a selected alkali halide, ammonium compound (e.g., NH_4, $N(CH_3)_4$, or a suitable ionic organic compound or ionic inorganic compound (e.g., $CaSO_4$, $Mg_m(PO_4)_n$). In one embodiment, a voltage difference is impressed, in a longitudinal direction or in a transverse direction, across an ionic liquid within the pore, and a varying ionic current through the pore, or a varying electron current across the pore (referred to collectively as an "electrical current") is measured in response to passage of each of an ordered sequence of polymer components, such as nucleotides in a nucleic acid or proteins, through the pore.

In one embodiment, the method includes steps of:

providing a pipette having a longitudinal axis and having a tapered region having a pore with a selected pore diameter in a range of 1-40 nanometers (nm);

providing a selected liquid in contact with an interior surface of the pore;

impressing a selected voltage difference across the selected liquid within the pipette pore substantially parallel to the pipette longitudinal axis direction, and providing an ionic current value induced in the selected liquid; and

passing an unknown polymer molecule, having a sequence of polymer components, through the pore, and determining a change in the ionic current signal induced by passage of each of the polymer components through the pore. In another embodiment, the voltage difference is impressed transversely, across the pore, and a transverse electronic current, induced in response to passage of each of the polymer components through the pore, is measured.

In another embodiment, a method for producing the pore includes steps of:

heating a hollow cylinder of a selected pipette material, having first and second cylinder ends, having a longitudinal axis and having a selected initial inner diameter, with a selected heating source for at least one of first and second longitudinal locations for at least one of first and second selected time intervals;

translating one of the first and second cylinder ends relative to the other of the first and second cylinder ends during a selected third time interval that partly or wholly overlaps at least one of the first time interval and the second time interval; and

allowing the hollow cylinder to separate into at least first and second pipettes and at least one of the first and second pipettes has a pore with a pore diameter in a range 1-40 nanometers (nm).

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. **1**A, **1**B and **1**C illustrate apparati for practicing the invention.

FIGS. **2**A, **2**B and **2**C graphical views of typical sequences of ionic or electron current values measured with no polymer component present (**2**A), and in response to passage of a polymer component through a pore (**2**B, **2**C).

FIGS. **3**, **4** and **5** are flow charts illustrating procedures for practicing the invention according to two embodiments.

FIGS. **6**A-**6**F graphically illustrate time variations that can be applied to an impressed voltage difference used in the invention.

FIGS. **7**A and **7**B illustrate formation of a pipette tip for use in the invention.

DESCRIPTION OF BEST MODES OF THE INVENTION

FIG. **1**A illustrates one embodiment of apparatus for measurement of longitudinal ionic current in practicing the inven-

tion. The system **11** includes a glass pipette **12**, having a longitudinal axis AA and having a tapered tip region **13**, with a pore **13***p* having a selected pore minimum inner diameter d(min) in a preferred range of 1-40 nm, or larger if necessary. A length of the interior surface of the tip region **13** is provided with a selected first liquid **14-1** including an ionizable molecule including an alkali halide (NaCl, KCl, NaBr, KBr, $MgCl_2$, $CaCl_2$, MgClBr, etc.), ammonium compound (e.g., NH_4, $N(CH_3)_4$, or a suitable ionic organic compound or ionic inorganic compound (e.g., $CaSO_4$, $Mg_m(PO_4)_n$). A portion of the pipette adjacent to the pore is immersed in a selected second liquid **14-2**, which may be, but need not be, the same as the first liquid **14-1**. The pore **13***p* has a pore length L(pore) in a selected length range. A voltage difference ΔV, having a value in a range 10-2000 milliVolts, is impressed substantially in the longitudinal axis direction across the liquid in the pore, and an ionic current value IC through the pore is measured by an electrical current measurement module **15** The voltage difference may, for example, be provided by a first electrode **17**A, positioned within the first liquid **14-1** in the interior surface of the pore **13***p*, and a second electrode **17**B, positioned within a "bath" **19** of the second liquid **14-2** surrounding the pore. One or both of the electrodes, **17**A and **17**B, may include Ag/AgCl or another substance known to provide reversible current and to have low offset voltage in ionic solutions.

FIG. **1**B, illustrating an embodiment for measuring transverse electronic current, is similar to FIG. **1**A, except that spaced apart electrodes, **18**A and **18**B, replacing the electrodes **17**A and **17**B, are arranged on or adjacent to a perimeter of the pore **13** and an electronic current flows from **18**A to **18**B in response to imposition of a voltage difference ΔV between these electrodes.

FIG. **1**C illustrates a different configuration of the pore **13***p*, according to the invention. In FIG. **1**C, different portions of an end **12***e* of the tip substantially face each other and define an effective pore length L(pore) that is approximately equal to a thickness of the pipette **12** at an end of the pipette. This configuration is preferred where the pore width d(min) is to be made as small as possible (e.g., less than or equal to 1.5 nm).

In one approach, an interior surface of the pore **13***p* is left uncoated in practicing the invention. Preferably, the interior surface of the pore **13***p* is coated or wetted or otherwise provided with a self-assembling monolayer ("SAM") **21** of a selected material that will manifest hydrogen bonding, van der Waals interaction and/or similar reversible, transient interactions with a class of polymers of interest. The SAM substance provided on an interior surface of the pore **13***p* may be octadecyltrichlorosilane ($C_{18}H_{37}SiCl_3$ or "OTS"), as discussed by Sagiv in "Organized Monolayers by Absorption. 1. Formation and Structure of Oleophobic Mixed Monolayers on Solid Surfaces," J. Amer. Chem. Soc. vol. 102 (1980) pp. 92-98, or may be another suitable substance that will interact with a polymer components passing through the pore **13***p* and allow measurement of a modulated ionic current signal or electron current signal that is characteristic of a particular polymer component. Other SAM substances that may be used include alkylsiloxane monolayers, alkylsilanes, trimethoxysilanes, mono-, di- and tri-chlorosilanes, octadecylsilanes, organochlorosilanes, aminosilanes, perfluorodecyltrichlorosilanes and aminopropylethoxysilanes.

From another perspective, a stable SAM can be formed using sulfur-containing absorbates on gold, chlorosilanes or alkoxysilanes on glass, and fatty acids on a metal oxide surface.

As used herein "SAM" includes an array of substantially identical molecules (e.g., containing a silane component) covalently attached to a glass surface and oriented substantially perpendicular to the surface, which interact, without permanent bonding, with a selected group of one or more solution molecules that pass near the SAM array. A SAM may be used to provide transient interactions with a polymer ubit passing through a nanopore and/or may be used to tailor the effective longitudinal and/or transverse dimensions (diameter, etc.) of a nanopore.

Formation of self-assembling monolayers on a gas or liquid interface were first reported by Langmuir in J. Am. Chem. Soc., vol. 39 (1917) 1848, and were first shown to be formable on a solid surface by Blodgett, J. Am. Chem. Soc., vol. 57 (1935) 1007. Spontaneous formation of a SAM on a solid substrate was first demonstrated by Bigelow, Pickett and Zisman, J. Colloid Interface Sci., vol. 1 (1946) 513 Chechik, Crooks and Stirling, in "Reactions and Reactivity in Self-Assembled Monolayers," Advanced Materials, 2000, 1161-1171, define a SAM as a monomolecular film of surfactant, formed spontaneously on a substrate upon exposure to a surfactant solution and provide a general review of use of SAMs. A SAM may be disordered or close packed, depending on the degree of wetting of the substrate surface. As noted by Chechik et al, ibid, a SAM is ideally suited as a scaffold to graft a polymer onto solid surfaces, because of the high density of functional groups, relatively small number of defects, and a well defined structure. A molecule that is part of a SAM may be terminated by various functional groups, such as OH and amines.

A second example of an SAM is (16-Mercapto)hexadecanoic acid (MHA) on a gold substrate, as studied and reported by J. Lahann et al in Science, vol. 239 (2003) pp 371-374. MHA includes hydrophobic chains capped by hydrophilic carboxylate end groups. Cleavage of the carboxylate end groups provides a low density SAM of hydrophobic chains. Application of a small electrical potential or voltage difference (e.g., $-1054\ mV \leqq V \leqq +654\ mV$ to the negatively charged carboxylate groups provides an attractive force that causes a conformation change in the hydrophobic chains, whereby an all-trans conformation becomes partly trans and partly gauche conformation, with substantial qualitative and quantitative changes in associated sum-frequency generation (SFG) spectroscopic variations associated with the different conformations. The conformational changes are reversible so that removal of the applied electrical potential causes a return to the relatively featureless SFG spectroscopic variations associated with the original hydrophobic chain conformations (all trans). Viewed from another perspective, change in hydrophobic chain conformations associated with a specific change, such as a variation in translocation associated with passage of different polymer units through a nanopore in which a very thin layer of MHA on gold is provided, would cause a measurable change in electrical current or change in electrical potential (tens to hundreds of millivolts) associated with passage of each (different) polymer unit.

In the absence of passage of a polymer component through the pore, a steady ionic current through the pore (or electronic current across the pore) will develop in response to impressing a small voltage difference in a longitudinal (or transverse) direction, as shown graphically in FIG. **2**A.

An ordered sequence of polymer components, such as a nucleic acid (e.g., DNA or RNA) is passed through the pore **13***p*, allowing development of a sequence of changing ionic current values, as illustrated in FIGS. **2**B and/or **2**C. In FIG. **2**B, passage of each polymer component through the pore **13***p* is assumed to produce an approximately square wave signal,

having an approximately constant characteristic amplitude for a small time interval that corresponds to the time required for that polymer component to pass through the tip. In FIG. **2**C, passage of each polymer unit through the pore **13***p* is assumed, more realistically, to produce a signal having a characteristic, time varying signal shape, a characteristic average amplitude and a characteristic shape parameter, for a small time interval that corresponds to the time required for that polymer component to pass through the tip.

Where one or more polymer components passes through the pore, translocation will cause the steady ionic current shown in FIG. **2**A to change with time in response to passage of the polymer component through the pore and the accompanying translocation. If, for example, the polymer sequence is a nucleic acid, such as DNA (alternatively, RNA), each nucleotide will contain one of the four bases adenine (A), cytosine (C), guanine (G) and thymine (T) (alternatively, adenine, cytosine, guanine and uracil (U) for RNA). Ideally, each of the four bases (for DNA or for RNA) will produce a distinguishable change in ionic current signal as that nucleotide passes through the pipette tip, as suggested in FIG. **2**B or FIG. **2**C.

Under the influence of an applied voltage difference, negatively charged nucleotides or other polymer units are driven through the pore, and a polynucleotide strand can thus be threaded from one side of a lipid biolayer to the other. A steady electrical current that is present in the pore in the absence of a polymer unit is partly occluded during translocation. In principle, polymer units of different lengths can be distinguished from each other by translocation duration, and several homopolymers of different composition can be distinguished based on characteristic levels of electrical current reduction.

FIG. **3** is a flow chart of a procedure for practicing the invention. In step **31** of FIG. **3**, a pipette, having a longitudinal axis and having a tapered tip with an associated pore having a selected pore minimum inner diameter d in a preferred range (e.g., d=1-40 nm) is provided, and a selected self-assembling monolayer (SAM) is optionally provided on some portion of the pore surface. In step **32**, a selected first liquid containing ions is provided in the interior surface of the pore, preferably containing an alkali halide, ammonium compounds (e.g., NH_4, $N(CH_3)_4$, or a suitable ionic organic compound or ionic inorganic compound (e.g., $CaSO_4$, $Mg_m(PO_4)_n$). More generally, the selected first liquid may be any solution that provides a concentration p of ions at least equal to a threshold value ρ(ion;thr), for example, $\rho(\text{ion;thr}) \geqq 10^x\ \text{cm}^{-3}$. The liquid may include the polynucleotide or other polymer that is to be identified. In step **33**, a voltage difference having a value in a range ΔV=10-2000 milliVolts is impressed on the liquid in the pore, in a direction substantially parallel to the pipette longitudinal axis. If the polymer has a net electrical charge, the polarity of the voltage difference is chosen to induce the polymer to pass through (or across) the pore. In step **34**, ordered components in a polymer (unknown) are sequentially passed through the pore, and each of a sequence of changes in ionic current signals is measured, resulting in a sequence of measured values such as the sequences shown in FIG. **2**B or FIG. **2**C.

In step **35** (optional), the sequence of changes in measured ionic current signals CIC(t;meas) is compared, one-by-one or in consecutive groups, with reference change signals CIC(t;ref;n), numbered n=1, . . . , N (N≧2) in a reference signal database. Each reference change signal corresponds to a reference polymer component. In step **36** (optional), each polymer component (e.g., a nucleotide containing a particular base) in the unknown sequence is assigned to the reference polymer component having a reference ionic current change signal that is most similar, in some quantitative sense, to the measured (changes in) ionic current change signal. Optionally, steps **35** and **36** are performed off-line

The signal comparison step **35** is optionally implemented as follows. The ionic current change signal CIC(t;meas) for the unknown polymer sequence is measured at a sequence of time values t_m, producing a sequence of measured ionic current change values $\{CIC(t_m;\text{meas})\}_m$. (m=1, . . . , M; M≧2) This sequence of measured ionic current change values is compared with a reference sequence (n) of ionic current change signal values $\{CIC(t_m+\tau(n);\text{ref};n)\}_m$, where τ(n) is a selected time shift that may vary with the reference number n being considered, by computing an error value

$$\epsilon(n)=\{w_m|CIC(t_m;\text{meas})-CIC(t_m+\tau(n);\text{ref};n)|^p\}^{1/p},\quad m=1\ \ldots\ M \tag{1}$$

where $\{w_m\}_m$ is a selected sequence of non-negative weight values (at least one positive) and p is a selected positive number (e.g., p=1, 1.6 or 2). Reference ionic current change signals $CIC(t_m+\tau;\text{ref};n)$ for which the error satisfies ϵ(n)>ϵ(thr), where ϵ(thr) is a selected positive threshold value, are discarded and not considered further for this measured ionic current change value sequence $\{CIC(t_m;\text{meas})\}_m$. When at least one error value satisfies ϵ(n)≦ϵ(thr), the "surviving collection"

$$SC=\{CIC(t_m+\tau(n);\text{ref};n)|\epsilon(n)\leqq\epsilon(\text{thr})\} \tag{2}$$

of all reference signals with error values that satisfy the inequality ϵ(n)≦ϵ(thr), are considered, and the reference ionic current change signal $CIC(t_m+\tau(n);\text{ref};n)$ that provides the smallest error ϵ(n) is assigned to the unknown polymer unit. When the surviving collection SC is an empty set, because no error value satisfies ϵ(n)≦ϵ(thr), the system assigns a selected symbol, such as UNK, to this polymer unit.

The comparison procedure can be summarized in a flow chart in FIG. **4**. In step **41**, the error ϵ(n), defined as in Eq. (1) or in another suitable manner, for each reference change signal $CIC(t_m+\tau(n);\text{ref};n)$ in the database is computed. In step **42**, the surviving collection SC of reference signals is determined. In step **43**, the system determines if SC is an empty set. If the answer to the query in step **43** is "yes," the system assigns a special symbol (e.g., UNK) to the corresponding measured ionic current value in step **44**, indicating that no reference change signal $IC(t_m+\tau(n);\text{ref};n)$ is sufficiently similar to the measured ionic current change signal. If the answer to the query in step **43** is "no" (SC is non-empty), the system identifies, in step **45**, each reference change signal $CIC(t_m+\tau(n);\text{ref};n=n')$ for which the corresponding error satisfies

$$\epsilon(n')=\min_{1\leqq n\leqq N}\epsilon(n). \tag{3}$$

In step **46**, the system identifies at least one reference polymer component, for which the corresponding reference change signal $CIC(t_m+\tau(n');\text{ref};n')$ is in the surviving collection SC, with the unknown polymer component whose signal was measured.

FIG. **5** is a flow chart of an alternate procedure for practicing the invention, using a transverse voltage difference. In step **51** of FIG. **5**, a pipette, having a longitudinal axis and having a tapered tip with an associated pore having a selected pore minimum inner diameter d in a preferred range (e.g., d=1-40 nm) is provided, where a selected self-assembling monolayer is optionally provided on an interior surface of the pore. In step **52**, the pore interior is provided with a selected first liquid, preferably containing an alkali halide, ammonium

compounds (e.g., NH_4, $N(CH_3)_4$, or a suitable ionic organic compound or ionic inorganic compound (e.g., $CaSO_4$, $Mg_m(PO_4)_n$), so that the first liquid is present within the pore. More generally, the selected liquid may be any solution that provides at least a concentration ρ of electrons at least equal to a threshold value ρ(ion;thr), for example, $\rho(\text{ion;thr}) \geqq 10^x\ \text{cm}^{-3}$. In step **53**, a voltage difference having a value in a range ΔV=10-2000 milliVolts, or more if desired, is impressed on the first liquid in the pore, in a direction substantially transverse to the pipette longitudinal axis. In step **54**, a polymer sequence (unknown) is sequentially passed through the pore, and each of a sequence of electron current change signals is measured, resulting in a sequence of measured values such as the sequences shown in FIG. **2**B or FIG. **2**C. The electron signals resulting from imposition of the transverse voltage difference are likely to be different from the corresponding ionic current signals resulting from imposition of a longitudinal voltage difference.

In step **55** (optional), the sequence of measured electron current change signals $CEC(t_m;\text{meas})$ is compared, one-by-one or in consecutive groups, with reference change signals $CEC(t_m+\tau(n);\text{ref};n)$, numbered n=1, . . . , N' (N'≧2) in a reference signal database. In step **56** (optional), each polymer unit (e.g., a nucleotide containing a particular base) in the unknown sequence is assigned to the reference polymer component having a reference electron current value that is most similar to the measured electron current signal. Step **55** may, for example, be implemented by analogy with implementation of step **35** in FIG. **3**, with electronic change signals, $CEC(t_m;\text{meas})$ and $CEC(t_m+\tau(n);\text{ref};n)$ replacing the corresponding ionic current change signals in Eqs. (1) and (2).

The voltage difference amplitude ΔV(t), impressed longitudinally or transversely across the selected liquid, may be substantially uniform in time, as illustrated in FIG. **6**A, may be substantially monotonically increasing in time (FIG. **6**B), may be substantially monotonically decreasing in time (FIG. **6**C), may be substantially a step function in time (FIG. **6**D), may vary substantially sinusoidally in time (FIG. **6**E), may vary substantially trapezoidally in time (FIG. **6**F), with temporal length segments τ1, τ2 and τ3, or may have another suitable time varying shape. The trapezoidal variation shown in FIG. **6**F includes a triangular variation, in which the middle segment has length τ2=0.

A tip region of a pipette (quartz glass, aluminosilicate glass, borosilicate glass or other suitable glass) having an appropriate minimum inner diameter may be formed using the following procedure, illustrated in FIGS. **7**A and **7**B. A selected middle region **73**, having a preferred length LH in a range 0.1-2 cm or more, of a pipette **71** with a hollow core is heated or otherwise receives substantial thermal energy, using a laser, infrared source or a heated metal filament **75** and (optional) associated focusing system **76**, at one or more locations, x=x1, x=x2, etc., for one, two or more time intervals, of length Δt1, Δt2, etc. The time intervals may partly or wholly overlap or may be isolated from each other. As the heating or irradiation continues, one or both of first and second ends, **77-1** and **77-2**, of the pipette is pulled with a selected force F, optionally 10^1-10^7 dynes or more, or at a selected displacement rate, v of a few mm/sec, so that the first and second ends are displaced relative to each other. The pipette **71** separates into two pipette segments, **71-1** and **71-2**, in the (last) heating cycle, and at least one of the two resulting pipette segments has a hollow core (a pore) with a pore minimum inner diameter d(min). Tip parameters (thickness, nanopore diameter, nanopore length, etc.) can be partly controlled by appropriate choice of one or more of the parameters heating rate, LH, Δt(irr), F and/or v.

Suitable applications of the invention, using ionic current or electronic current, include the following: (1) counting of genomic or non-genomic fragments, by identification of a first end and/or a second end of each fragment that passes through a nanopore; (2) identification of locations of single strand segments and double strand segments in a "mixed" DNA sequence passing through a nanopore; (3) discrimination between single strands and double strands of DNA passing through a nanopore; and (4) identification of individual nucleotides in single strand DNA passing through a nanopore; (5) identification of corresponding base pairs (e.g., cytosine-guanine, adenine-thymine and adenine-uracil) in a double strand DNA or RNA passing through a nanopore, and (6) estimation of polymer component length by correlation with length of time interval for translocation.

What is claimed is:

1. A method of fabricating a nanopore, the method comprising:

heating a hollow cylinder of a pipette material, comprising primarily at least one of quartz glass, aluminosilicate glass and borosilicate glass, by a process comprising use of at least one of a laser, an infrared light source and a heated metal for heating one or more locations on the cylinder for a first time interval, the hollow cylinder having first and second cylinder ends, having a longitudinal axis and having a non-zero initial inner diameter; and

applying a machine controlled translation force to translate at least one of the first and second cylinder ends relative to the other of the first and second cylinder ends by a change in end-to-end separation distance no greater than about 2 cm during a second time interval that partly or wholly overlaps the first time interval, in order to encourage the hollow cylinder to separate into at least first and second pipettes, each with a corresponding nanopore, with at least one pore diameter in a range of 1-40 nanometers (nm) and with at least one pore length no greater than about 2 cm.

2. The method of **1**, further comprising choosing said heating source from a group of heating sources consisting of a laser, an infrared light source, and a heated metal.

3. The method of claim **1**, further comprising:

providing a selected liquid in contact with an interior surface of said pore;

impressing a non-zero voltage difference across the selected liquid within said pore approximately parallel to a longitudinal axis direction of said cylinder, and determining at least one of an electrical current value and an ionic current value induced in the selected liquid; and

passing a polymer molecule, having a sequence of polymer components, through said pore in a first direction, determined with reference to the longitudinal axis direction, and determining at least one of an electrical current signal and an ionic current signal induced by passage of each of the polymer components through said pore.

4. The method of claim **3**, further comprising selecting material for said pipette from a group of materials including quartz glass, aluminosilicate glass and borosilicate glass.

5. The method of claim **3**, further comprising passing said polymer sequence through said pore at an average rate in a range of 1-1000 polymer components per msec.

6. The method of claim **3**, further comprising choosing said polymer sequence to be a nucleic acid sequence including the bases adenine, cytosine and guanine and at least one of the bases thymine and uracil.

7. The method of claim 3, further comprising selecting said voltage difference from a group of time-dependent differences including a difference that (i) is approximately uniform in time; (ii) increases monotonically with time; (iii) decreases monotonically with time; (iv) is a step function in time; (v) varies sinusoidally with time; and (vi) varies trapezoidally with time.

8. The method of claim 3, further comprising choosing said selected liquid to include at least one of an alkali halide, an ammonium compound, an ionic organic compound and an ionic inorganic compound.

9. The method of claim 1, further comprising providing a self-assembling monolayer of a selected substance on a selected portion of said interior surface of said pore.

10. The method of claim 9, further comprising choosing said self assembling monolayer to include at least one of: (i) octadecyltrichlorosilane on glass and (ii) (16-Mercapto) hexadecanoic acid on a gold substrate.

11. The method of claim 1, further comprising:

providing a selected liquid in contact with an interior surface of said pore;

impressing a non-zero voltage difference across the selected liquid within said pore transverse to a longitudinal axis direction of said cylinder, and determining an ionic current value induced in the selected liquid; and

passing a polymer molecule, having a sequence of polymer components, through said pore in a first direction, determined with reference to the longitudinal axis direction, and determining an ionic current signal induced by passage of each of the polymer components through said pore.

12. The method of claim 11, further comprising passing said polymer sequence through said pore at an average rate in a range of 1-1000 polymer components per msec.

13. The method of claim 11, further comprising choosing said polymer sequence to be a nucleic acid sequence including the bases adenine, cytosine and guanine and at least one of the bases thymine and uracil.

14. The method of claim 11, further comprising selecting said voltage difference from a group of time-dependent differences including a difference that (i) is substantially uniform in time; (ii) increases monotonically with time; (iii) decreases monotonically with time; (iv) is a step function in time; (v) varies sinusoidally with time; and (vi) varies trapezoidally with time.

15. The method of claim 11, further comprising choosing said selected liquid to include at least one of an alkali halide, an ammonium compound, an ionic organic compound and an ionic inorganic compound.

16. The method of claim 1, further comprising providing a self-assembling monolayer of a selected substance on a portion of said interior surface of said pore.

17. The method of claim 16, further comprising choosing said self assembling monolayer to include at least one of: (i) octadecyltrichlorosilane on glass and (ii) (16-Mercapto) hexadecanoic acid on a gold substrate.

18. The method of claim 1, further comprising applying said machine controlled translation force in a range of between 10 dynes and 10 million dynes in a direction corresponding to said longitudinal axis.

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